# Center for Veterinary Biologics
# and
# National Veterinary Services Laboratories
# Testing Protocol

## Supplemental Assay Method for Bacterial Count of *Pasteurella multocida*, Avian Isolates, Vaccines

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## Table of Contents

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# 1. Introduction

## 1.1 Background

This is a Supplemental Assay Method (SAM) for the titration analysis of *Pasteurella multocida*, avian isolates, vaccine, live culture. It determines the colony-forming units (CFU) in final container samples as prescribed by the Code of Federal Regulations, Title 9 (9 CFR) Part 113.70. This method uses tryptose agar with 5% bovine blood and tryptose broth as a diluent.

## 1.2 Keywords

*Pasteurella multocida*; potency; viable count

# 2. Materials

## 2.1 Equipment/instrumentation

**2.1.1** Vortex mixer

**2.1.2** Colony counter

**2.1.3** Inoculum spreader

**2.1.4** Bunsen burner

**2.1.5** Disposable syringes and needles--appropriate sizes

**2.1.6** Sterile disposable pipettes--appropriate sizes

**2.1.7** Sterile screw-capped (sc) culture tubes, 20 x 150 mm

**2.1.8** Pipetting aid

**2.1.9** 35° ± 2°C incubator

**2.1.10** Biosafety cabinet

**2.1.11** Gloves and lab coat or frock

**2.1.12** 4 x 4 sterile gauze pads

**2.1.13** Test tube rack

### 2.2 Reagents/supplies

**2.2.1** Tryptose broth **(Section 9.1)--**National Veterinary Service Laboratories (NVSL) Media No. 10404

**2.2.2** Tryptose agar with 5% bovine blood **(Section 9.2)**--NVSL Media No. 10218 or as stated in the biologics manufacturer's Outline of Production (OP)

**2.2.3** *P. multocida* reference culture (American Type Culture Collection 11039)

**2.2.4** 70% ethyl alcohol

**2.2.5** Sterile water in serum vials--volumes determined by referring to the biologics manufacturer's OP or as stated on the vaccine vial

## 3. Preparation for the test

### 3.1 Personnel qualifications/training

The personnel must have experience or training in this SAM. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

## 3.2 Preparation of equipment/instrumentation

**3.2.1** Turn on the biosafety cabinet 60 min before use and turn off after use.

**3.2.2** Monitor the incubator daily for temperature according to the current version of GDOCSOP0001.

**3.2.3** Monitor the freezers and coolers used for storing samples daily for temperature according to the current version of GDOCSOP0003.

## 3.3 Preparation of reagents/control procedures

**3.3.1** Warm the samples and reference culture to room temperature before rehydrating to the appropriate volume.

**3.3.2** Prepare *P. multocida* reference control samples according to the current version of STRPP0001.

**3.3.3** Negative and Positive Controls: Incubate 2 uninoculated plates of tryptose agar with 5% bovine blood with test sample plates as negative control plates. *P. multocida* reference culture (positive control) is diluted the same as the test samples, but plated depending on the titer found in **Section 3.3.2.**

**3.3.4** Store plates used for making counts at refrigerator temperature. Plates to be used for counts are placed in a 35° ± 2°C incubator overnight prior to use or allowed to dry in a biosafety cabinet before use. At the time of use, plates are no more than 14 days old.

## 3.4 Preparation of the sample

Samples of *P. multocida* vaccines are received from the Biological Materials Processing Section (BMPS) according to the current version of STSOP0001.

## 4. Performance of the test

**4.1** Remove 2 vials of product to be tested and 1 vial of *P. multocida* reference control sample from the freezer or cooler storage and allow to warm to room temperature.

**4.2** Disinfect the caps with 70% ethyl alcohol. If needed, rehydrate the vials with the accompanying diluent or sterile water. Allow the contents of the vials to reconstitute for at least 5 min. Shake the vials by inversion until thoroughly mixed.

**4.3** Prepare a tenfold dilution series of the first vial of the product by setting up a rack of 20 x 150-mm sc tubes and pipetting 9 ml of tryptose broth into each tube using a 10-ml pipette. Label the tubes $10^{-1}$ to $10^{-x}$ as needed.

**4.4** Transfer 1 ml of sample from **Section 4.2** into the first tube of tryptose broth using a pipette. Cap the tube and vortex. Continue the dilution series by using a pipette to transfer a 1-ml sample to the next tube, labeled $10^{-2}$. Repeat this method using a sterile pipette for each transfer until the required number of serial tenfold dilutions (as determined by the release titer in the firm's OP) is attained.

**4.5** Deposit 0.1 ml of the sample from the last 3 dilution points of the dilution series for the product onto the surface of media in **Section 2.2.2** using a pipette. Label the 3 plates for each dilution point with the sample number or name, vial number, and dilution.

**4.6** Use a sterile spreader to evenly distribute the inoculum over the surface of the agar medium.

**4.7** Repeat **Sections 4.3 through 4.6** with the second vial of product.

**4.8** Repeat **Sections 4.3 through 4.6** with the reference or positive control vial of *P. multocida*. Prepare plates at the 3 reference control dilutions determined from **Section 3.3.2.**

**4.9** Use 2 uninoculated plates of media as negative controls.

**4.10** Invert all plates and incubate at 35° ± 2°C for 24 hr. After incubation, count plates from each series that contains 30 to 300 CFU. Multiply the CFU by the dilution factor and determine the CFU per dose for the dilution series. Determine the mean CFU per dose for the number of vials tested.

## 5. Interpretation of the test results

**5.1** If on the initial test the CFU per dose is equal to or exceeds the required minimum as written in the firm's OP, the serial or subserial is satisfactory (SAT) for bacterial count without additional testing.

**5.2** If on the initial test the CFU per dose is less than the required minimum release titer as written in the firm's OP, the serial or subserial may be retested using 4 new vaccine samples. A comparison of the firm's OP method to this SAM shall be done. If the retest (RT) is not done, the serial or subserial is unsatisfactory (UNSAT). If on the RT, the average count of the 4 vaccine samples is less than the required minimum, the serial or subserial is UNSAT.

**5.3** If on the RT of the 4 new vials of vaccine, the average count is equal to or greater than the required minimum release titer, the serial or subserial is SAT.

**5.4** If on the initial test the reference culture or positive control culture is not within the titer range determined in **Section 3.3.2**, but the serial being tested has a SAT result, the serial or subserial is a no test (NT) for bacterial count without additional testing. If the

reference culture is not within the titer range and the serial being tested is below the minimum release titer, the serial is retested using 2 new vaccine samples. If on the initial test there is growth on the negative control plates, the serial or subserial is a NT for bacterial count without additional testing.

## 6. Report of test results

**6.1** Record the CFU per dose along with the final conclusion for the product tested on the log book record sheet (STFRM00PT) and the computer worksheet after calculating the CFU per dose and interpreting the results. Enter the results and conclusions into the computer under the ST test code 070-PT0 as stated in the current version of STSOP0021.

**6.2** Initial and date the log book record sheet and the computer worksheet. Forward all paperwork to the Cytology and Sterility Section supervisor or microbiologist to review and sign.

**6.3** Validate the test results as stated in the current version of STSOP0021 and file all paperwork appropriately.

## 7. Reference

Code of Federal Regulations, Title 9, Part 113.70, U.S. Government Printing Office, Washington, DC, 1999

## 8. Summary of revisions

This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.

## 9. Appendices

**9.1** NVSL Media Formulation No. 10404

Tryptose broth

| | |
|---|---|
| Tryptose broth | 26.0 g |
| $H_2O$ | 1000.0 ml |

Autoclave 20 min at 121°C.

**9.2** NVSL Media Formulation No. 10218

Tryptose agar with 5% bovine blood (defibrinated)

| | |
|---|---|
| Tryptose agar | 41.0 g |
| $QH_2O$ | 950.0 ml |

Autoclave 25 min at 121°C. Cool in waterbath at 56°C and add 50.0 ml defibrinated bovine blood.